Nrf2-Keap1 SIGNALING PATHWAY REGULATION DURING EARLY APOPTOSIS

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Nrf2 (Nuclear Factor-erythroid 2-related Factor 2) is the major regulator of cytoprotective responses to endogenous stresses caused by reactive oxygen species (ROS) and electrophiles[1]. Under basal condition, Nrf2 is sequestered in cytoplasm by its binding to Keap1, which functions as a substrate adaptor for Cul3 ubiquitin ligase complex leading to continuous ubiquitination of Nrf2 and its proteosomal degradation. The half life of Nrf2 in cells was shown to be no more than 30 min. Upon challenge with agents that produce oxidative stress, Nrf2 protein levels rapidly increase in the nucleus of affected cells through the disrupted of binding equilibrium between Keap1 and Nrf2. Nrf2 is translocated into nucleus and heteromerizes with members of small Maf protein family (MafG and MafF) and triggering the transactivation of a battery of cytoprotective genes, each containing at least one antioxidant response element (5´a/GTGAC/GNNNGCa/G-3') in their promoters[2]. Nrf2 is a double edge sword that is required for cellular protection, but accumulation of nuclear Nrf2 also leads to problems associated with decreased apoptosis/increased survival of damaged cells due to the cells can proliferate in high levels of oxidative stress avoiding the apoptosis process[3]. Since activation of apoptosis requires the accumulation of ROS, it is plausible that the Nrf2 antioxidant response pathway must be suppressed in order to trigger apoptosis[4]. In this study we evaluated if the Nrf2 activity has a regulation depending on citotoxicity caused by curcumin, for this reason we exposed EBV immortalized lymphocytes to a dose and time dependent treatment (dose: 5, 10, 15, 20 y 30uM) (time: 6,12,18 and 24 hour treatment with 15uM Curcumin) and we evaluated cell viability through trypan blue assay, live and dead kit, annexin V kit, caspase 3 and 9 processing as well as caspase 3 activity through PARP processing. We found a concentration and time of exposition in which the cell is engaged to die (15uM Curcumin 18-hour treatment). Besides that, we observed an increase in the total and nuclear Nrf2 protein level and an increase in mRNA in some of it target genes: *HMOX1, GCLM, GCS, TRX, NQO1, SQSTM1, GSTM3* and *GSR* at 12-hour treatment with 15uM of Curcumin. Meanwhile at 18-hour treatment when the cells are engaged to apoptosis the total and nuclear protein are lowered, as well as, mRNA levels of HMOX, GCLM, GCS and TRX diminished. Neverthless, the mRNA levels of NQO1 were increasing until 24-hour treatment. We can conclude that seems to be needed the down regulation of Nrf2 activity for the induction of apoptotic process under oxidative stress.

1Kansanen et al., Redox Biology 2013 1: 45-49

2. Geisman et al., OncoTargets and Therapy 2014 7: 1497–1518

3 Jaramillo and Zhang, Genes and development 2013 27: 2179-2191

4Villeneuve et al., Cell Cycle 2009 8(20): 3255-3256